have greatly reduced inflammatory pain sensation. More recently, mutations in *SCN11A* encoding human $Na_V1.9$ have been associated with either loss of pain perception, or familial episodic pain and painful peripheral neuropathy. Here we elucidate the functional consequences of a novel heterozygous $Na_V1.9$ mutation (L1302F) discovered in a female diagnosed with congenital insensitivity to pain (CIP). The mutation was stably expressed in ND7/23 cells and whole-cell currents were elicited with 50 ms pulses from −120 to +40 mV from a holding potential of −140 mV in the continuous presence of 150 nM TTX to block endogenous TTX-sensitive sodium currents. Cells expressing $Na_V1.9$-WT exhibited whole-cell current that peaked at −40 mV (31.2 ± 3.5 pA/pF, n=10) with a voltage-dependence of activation defined by V½ = −61.8 ± 1.5 mV and slope factor (k) of 6.1 ± 0.4 (n=10). By contrast, $Na_V1.9$-L1302F whole-cell current peaked at −70 mV (39.5 ± 8.7 pA/pF, n=12) and exhibited a significantly hyperpolarized voltage-dependence of activation (V½ = −86.6 ± 1.1 mV; k = 6.5 ± 0.4; n=12). These results initially appeared to indicate that the mutation potentiates channel function by enabling activation at more negative membrane potentials. However, when currents were recorded using a holding potential of −90 mV, mutant channel activity was reduced substantially by ~95% (1.5 ± 0.3 pA/pF @ −70 mV, n=7), whereas the WT channel had more preserved activity ~50% (15.6 ± 3.1 pA/pF @ −40 mV, n=6). These results suggest that the effect of this mutation is more likely a loss-of-function under physiological conditions, and this will reduce neuronal excitability leading to impaired pain sensation.

2913-Pos Board B343

Infant Sudden Death: Novel Mutations Responsible for Impaired Nav1.5 Channel Function

Jace Morganstein[1], Kundan Jana[1], Monique N. Foster[1], Tomoe Y. Nakamura[2], Thomas V. McDonald[3], Yingying Tang[4], **William A. Coetzee**[1].
[1]NYU School of Medicine, New York, NY, USA, [2]National Cerebral and Cardiovascular Center Research Institute, Suita, Osaka, Japan, [3]Albert Einstein College of Medicine, New York, NY, USA, [4]Office of Chief Medical Examiner, New York, NY, USA.

Sudden infant death syndrome (SIDS) is the leading cause of mortality in apparently normal infants. During 2008 to 2012, the New York City Office of Chief Medical Examiner (OCME) examined 274 cases of sudden unexplained death (SUD) of which 141 were infants below 1 year of age, with ~93% of these less than 6 months of age at the time of death. Several ion channelopathies were found during genetic screening. An African-American/Hispanic girl who died suddenly in her sleep at the age of 5 weeks carried two SCN5A mutations: c.5494 C>G and c.5830 C>T, which respectively introduces a missense mutation Q1832E and an early stop codon R1944X in the distal C-terminus of the cardiac Na^+ channel -subunit, Nav1.5. HEK-293 cells were transfected with cDNAs of wild-type Nav1.5, Nav1.5-Q1832E, Nav1.5Δ1944 (the C-terminal truncation) or a cDNA with both mutations (Nav1.5-Q1832E-Δ1944) and were subjected to whole-cell patch clamping. The peak Nav1.5-Q1832E current was reduced by almost 10-fold (e.g. at −20mV the wild-type Nav1.5 was 283 ± 49.1 pA/pF, n=8 cf. −31 ± 11.8 pA/pF, n=4, for Nav1.5-Q1832E, $p<0.001$), whereas Nav1.5Δ1944 and Nav1.5-Q1832E-Δ1944 currents were not significantly different from wild-type. The inactivation time constants were unaffected by any of the mutations (e.g. at −10mV, 1 and 2 respectively were 1.3 ± 0.15 and 7.7 ± 0.87 ms, n=8 for wild-type cf. 1.1 ± 0.21 and 7.5 ± 1.61 ms, n=4, for Nav1.5-Q1832E). No significant differences were observed for the time course of the recovery from inactivation or the voltage dependence of the activation and inactivation kinetic variables. Preliminary biotinylation experiments suggest that the Nav1.5-Q1832E surface expression was unaltered compared to wild-type, suggesting a defect independent of trafficking. In conclusion, the Q1832E mutation was sufficient to produce a severely dysfunctional Nav1.5 channel, which may have been contributing to the victim's sudden death.

2914-Pos Board B344

Biophysical and Molecular Analysis of the Sodium Current in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

Brian K. Panama, Robert J. Goodrow, Serge Sicouri, Charles Antzelevitch, Jacqueline A. Treat, **Jonathan M. Cordeiro**.
Experimental Cardiology, Masonic Medical Research Laboratory, Utica, NY, USA.

Background: Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been used for safety pharmacology and to investigate genetic diseases affecting cardiac ion channels. We examined I_{Na} in hiPSC-CMs and determined its contribution to action potentials (APs) recorded from monolayers of hiPSC-CMs.
Methods: Commercially available hiPSC-CMs were plated at high density to form monolayers or low density to yield single cells. AP recordings from monolayers were made using high resistance electrodes at 36°C. Whole cell patch clamp was used to record I_{Na} in single hiPSC-CMs at room temperature.
Results: AP recordings showed spontaneous activity with a maximum diastolic potential (MDP)= −69.2 ± 1.4 mV and upstroke velocity=41.9 ± 6.7 V/s. Application of tetrodotoxin resulted in a slowing of the AP rate but had little effect on AP upstroke or duration. In single hiPSC-CMs, a large I_{Na} was recorded when external Na^+ was reduced to 40 mM (73.6 ± 6.18 pA/pF). Recovery of I_{Na} (hp= 120 mV) was very fast; at hp= 80 mV, recovery of I_{Na} was slower and the size of peak I_{Na} was greatly reduced (27.0 ± 3.38 pA/pF). Molecular analysis showed that SCN5A was the predominate Na^+ channel subtype in both adult and iPSC-CMs. In adddition, we found that iPSC-CMs express both the fetal (exon 6A) and adult (exon 6) isoforms of SCN5A. Action potential clamp experiments showed that application of a ventricular or Purkinje cell waveform to the same hiPSC-CM elicited a large I_{Na} while application of a SA node waveform elicited no I_{Na}.
Conclusion: A large I_{Na} is present in hiPSC-CM but its contribution to the AP upstroke is minimal. The depolarized MDP coupled with the presence of phase 4 depolarization results in a take-off potential of −60.6 ± 1.7 mV which inactivates the majority of Na^+ channels.

2915-Pos Board B345

Nav1.5 C-Terminal Domains Influence Calcium Regulation of Fast Inactivation Separately from Calmodulin Interaction

Franck Potet[1,2], Svetlana Stepanovic[2], Sabina Kupershmidt[2], Alfred L. George, Jr.[1].
[1]Pharmacology, Northwestern University, Chicago, IL, USA, [2]Anesthesiology, Vanderbilt University, Nashville, TN, USA.

The cardiac sodium channel (Nav1.5) has a complex 'intracellular Ca^{2+} sensing apparatus' within its C-terminal domain (CTD) consisting of a partial EF-hand domain (CTD-EF) and a calmodulin (CaM) binding IQ motif (CTD-IQ). There are additional CaM binding motifs within the DIII-DIV linker. Variation in intracellular Ca^{2+} concentration influences the voltage-dependence of steady-state fast inactivation (SSI) by an unclear mechanism. Here, we mutated 16 key residues implicated in the interactions between CTD-EF and CTD-IQ, CTD-IQ and CaM, or DIII-DIV and CaM and explored their impact on NaV1.5 function and biochemistry. We used quantitative yeast-two-hybrid assays to measure effects of mutations on the interaction between the full length CTD on CaM, and evaluated SSI in high (1 μM free Ca^{2+}) and low $[Ca^{2+}]$i conditions. Using either BAPTA or HEDTA as chelator, 1 μM free $[Ca^{2+}]$i was sufficient to shift SSI (elicited by 50 ms prepulses) towards depolarized potentials. We observed that 3 of 5 CTD-IQ mutations (F1912A, A1924T and IQ/AA) strongly reduced the interaction between the CTD and CaM. However, these mutations did not affect the $[Ca^{2+}]$i effect on SSI suggesting that SSI $[Ca^{2+}]$i sensitivity does not depend on CaM binding to the CTD-IQ. Unexpectedly, single and combination mutations of the CTD-EF (L1786A, F1791A, Q1807A, L1862A, E1788A-D1790A-D1792A-E1799A, E1804A-D1802A) diminished the CTD-CaM interaction, and some of these mutations (L1786A, E1788A-D1790A-D1792A-E1799A, E1804A-D1802A) also suppressed the $[Ca^{2+}]$i effect on SSI. Indeed, the only mutations we studied that blunted the $[Ca^{2+}]$i effect on SSI were within the CTD-EF. These results suggest that the CTD-EF influences NaV1.5 $[Ca^{2+}]$i sensitivity and mutations in this domain can also alter the interaction of the CTD with CaM, but CaM interaction with the CTD-IQ is not required for the effect of intracellular Ca^{2+} on inactivation.

2916-Pos Board B346

CaMKII-Dependent Regulation of Cardiac Sodium Channel

Federica Farinelli, Deborah DiSilvestre, Peihong Dong, Yanli Tian, Gordon Tomaselli.
Cardiology, Johns Hopkins School of Medicine, Baltimore, MD, USA.

Voltage-gated Na^+ channels are key determinants of conduction, action potential profile and refractoriness in mammalian myocardium. Na^+ channels are regulated by a number of protein kinases and alterations in phosphorylation are associated with the phenotypic expression of inherited and acquired heart diseases. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylates $Na_V1.5$ at multiple sites in the domain I-II linker, with effects on channel gating. The CaMKII phosphorylated sites we identified by mass spectrometry (MS) include serines 459, 460, 484, 539, 571, 664, 667 and threonine 486 in the I-II linker and serines 1925, 1937 and 1969 in the carboxyl-Terminus (CT). In addition we evaluated the effect of CAMKII phosphorylation on $Na_V1.5$-S528A and $Na_V1.5$-R526H channels in which direct phosphorylation by PKA is abolished (Aiba, 2014). In Wild-type $Na_V1.5$ channels, acute exposure to CaMKII increased the peak current,